

Atty Dkt. No.: CLON-037CON
USSN: 09/839,696

In the claims:

Cancel Claims 1-13 and 18-37.

14. (Currently Amended) A method for synthesizing carboxymethylated aspartate agarose chelating resin, said method comprising:

- (a) forming oxirane-agarose;
- (b) conjugating aspartic acid to ~~said oxirane-agarose to produce aspartate agarose;~~
- (c) ~~carboxymethylating said aspartate agarose to produce carboxymethylated aspartate agarose; and~~
- (d) ~~complexing said carboxymethylated aspartate agarose with a metal ion other than Ca²⁺ washing said aspartic acid-oxirane-agarose conjugate to remove extraneously bound metals using a high ionic strength solution.~~

15. (Original) The method, according to claim 14, wherein said conditions for oxirane-agarose formation comprise carrying out the formation at about room temperature, overnight, adjusting to about pH 7.0.

16. (Currently Amended) The method, according to claim 14, wherein said temperature control conditions for conjugating aspartic acid to said oxirane-agarose comprises ~~mixing at less than about 25 °C, reacting said oxirane-agarose and said aspartic acid~~ at about 80°C for 4 hours, then cooling to room temperature overnight.

17. (Currently Amended) The method, according to claim 14, wherein said ~~method further comprises washing said aspartate-agarose to remove extraneously bound metals~~ washing step (c) comprises use of a solution of at least 7.5% sodium hydroxide.

Please enter the following new claims:

Atty Dkt. No.: CLON-037CON
USSN: 09/839,696

38. (New) The method according to claim 14, wherein said metal ion is a transition metal ion.

39. (New) The method according to claim 14, wherein said transition metal ion is a third-block transition metal ion.

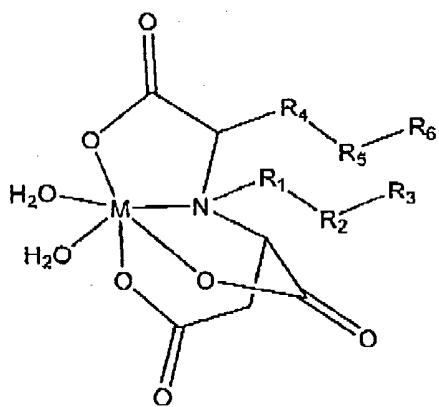
40. (New) The method according to claim 39, wherein said transition metal ion is selected from the group consisting of Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} .

41. (New) The method according to claim 40, wherein said transition metal ion is Co^{2+} .

42. (New) The method according to claim 38, wherein said transition metal is complexed to said carboxymethylated aspartate agarose in an octahedral geometry.

43. (New) The method according to claim 38, wherein said complex offers two available valencies.

44. (New) The method according to claim 14, wherein said carboxymethylated aspartate agarose chelating resin is described by the formula:



Atty Dkt. No.: CLON-037CON
USSN: 09/839,696

wherein R₄-R₅-R₆ = H;

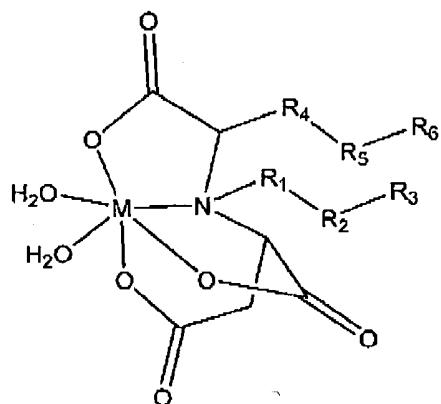
M = transition metal ion in a 2+ oxidation state with a coordination number of 6;

R₁ = a linking arm connecting the nitrogen atom of CM-Asp with R₂;

R₂ = a functional linking group through which CM-Asp linking arm R₁ is connected to R₃;
and

R₃ = an agarose matrix.

44. (New) The method according to Claim 14, wherein said carboxymethylated aspartate agarose chelating resin is described by the formula:



wherein R₁-R₂-R₃ = H;

M = transition metal ion in a 2+ oxidation state with a coordination number of 6;

R₄ = a linking arm connecting the methylene carbon atom of the carboxymethyl group of CM-Asp with R₅;

R₅ = a functional linking group through which CM-Asp linking arm R₄ is connected to R₆;
and

R₆ = an agarose matrix.

45. (New) A method for synthesizing a chelating matrix, said method comprising:

(a) reacting an ω -monoprotected α,ω -diamino acid with maleic acid to form a Michael addition product;

Atty Dkt. No.: CLON-037CON
USSN: 09/839,696

- (b) deprotecting the ω -amino functional group of the Michael addition product;
- (c) attaching the Michael addition product to a polymer matrix to produce said chelating matrix.

46. (New) The method according to claim 45, wherein the protecting group on said protected amino group is a benzyloxycarbonyl group.

47. (New) The method according to claim 45, wherein said polymer matrix is a matrix suitable for use in affinity or gel chromatography.

48. (New) The method according to claim 45, wherein said polymer matrix is selected from the group consisting of agarose, cross-linked agarose, polystyrene, SEPHAROSE, and nylon.

49. (New) The method according to claim 45, wherein said ω -monoprotected α,ω - diamino acid is N₆-Carbobenzyloxy-L-lysine.

50. (New) The method according to claim 45, wherein said method further comprises complexing said chelating matrix with a metal ion other than Ca²⁺.

51. (New) The method according to claim 50, wherein said metal ion is a transition metal ion.

52. (New) The method according to claim 51, wherein said transition metal ion is a third-block transition metal ion.

52. (New) The method according to claim 52, wherein said transition metal ion is selected from the group consisting of Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺.

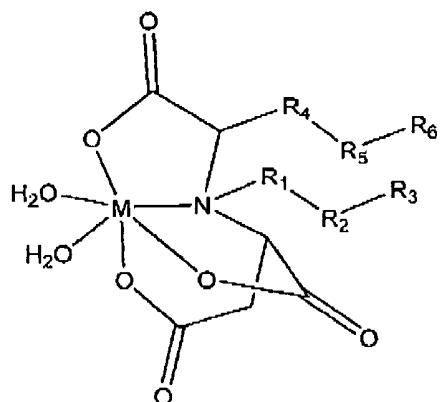
53. (New) The method according to claim 52, wherein said transition metal ion is Co²⁺.

Atty Dkt. No.: CLON-037CON
USSN: 09/839,696

54. (New) The method according to claim 51, wherein said transition metal is complexed to said carboxymethylated aspartate agarose in an octahedral geometry.

55. (New) The method according to claim 51, wherein said complex offers two available valencies.

56. (New) The method according to claim 50, wherein said chelating matrix loaded with a metal ion is described by the formula:



wherein R₄-R₅-R₆ = H;

M = transition metal ion in a 2+ oxidation state with a coordination number of 6;

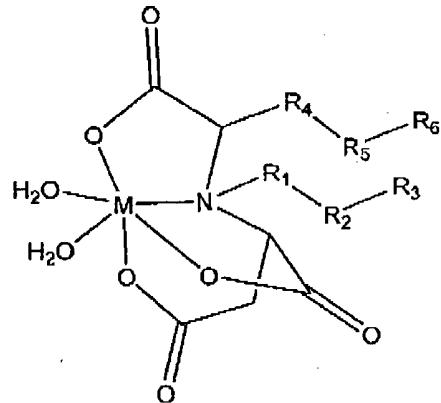
R₁ = a linking arm connecting the nitrogen atom of CM-Asp with R₂;

R₂ = a functional linking group through which CM-Asp linking arm R₁ is connected to R₃;

and

R₃ = a polymer matrix.

57. (New) The method according to Claim 50, wherein said chelating matrix loaded with a metal ion is described by the formula:

Atty Dkt. No.: CLON-037CON
USSN: 09/839,696

wherein R₁-R₂-R₃ = H;

M = transition metal ion in a 2+ oxidation state with a coordination number of 6;

R₄ = a linking arm connecting the methylene carbon atom of the carboxymethyl group of CM-Asp with R₅;

R₅ = a functional linking group through which CM-Asp linking arm R₄ is connected to R₆;
and

R₆ = a polymer matrix.